Clin. exp. Immunol. (1989) **77**, 1–6

Human immunodeficiency virus (HIV) infection in haemophiliacs: long-term prognostic significance of the HIV serologic pattern

K. RAŠKA JR., H. C. KIM, K. RAŠKA III, E. MARTIN, J. RASKOVA & P. SAIDI
Departments of Pathology and Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School Piscataway and New Brunswick, NJ

(Accepted for publication 17 April 1989)

SUMMARY

To identify markers of prognostic value in the course of HIV disease, immunologic parameters and profiles of HIV antibodies and antigen were studied in 60 haemophiliacs. The 43 HIV-seropositive subjects were followed prospectively over a 4 year period with a retrospective analysis as well of their frozen plasma for HIV markers. This group had a significant decrease in number of helper/inducer T lymphocytes as compared with 17 HIV seronegative subjects. The degree of changes correlated with the stage of disease, with the most severe depletion of CD4 cells in those who developed AIDS. Counts of B cells and platelets were also lower in HIV-infected haemophiliacs. Ten out of 12 AIDS patients had undetectable antibodies to HIV p24 antigen; low levels of p24 antibody were also seen in six out of 15 subjects with lymphadenopathy (CDC stage III), but in only two out of 16 asymptomatic subjects (CDC stage II). Sustained HIV p24 antigenaemia (> 30 pg/ml) was seen in 10 AIDS patients, in five subjects with lymphadenopathy and in two asymptomatic haemophiliacs. Initial HIV serologic profiles, obtained when all patients were asymptomatic, were highly predictive for progression of the HIV infection: the initial pattern of low anti-p24 antibody and positive p24 antigenaemia conferred the worst prognosis, with all patients in this group developing ARC or AIDS within 36 months, whereas an initial high level of anti-p24 without p24 antigenaemia was associated with relatively the best prognosis. Of such subjects, 58% have remained clinically asymptomatic after 48 months of the study ($P < 0{\cdot}00001$). The serologic profile of HIV antibody pattern and HIV antigen in haemophilic patients thus already provides important prognostic information at an early stage of HIV infection.

Keywords AIDS haemophilia antibodies to HIV HIV p24 antigens

INTRODUCTION

Hemophiliacs are a well-identified group with a high prevalence of HIV infection (Safai *et al.*, 1984; Centers for Disease Control (CDC), 1986). With overall seroprevalence in adults ranging from 75 to 90%, due to multiple past exposures to contaminated batches of coagulation factor concentrate (Hilgartner, 1987), AIDS now represents the leading cause of death among haemophiliacs (Johnson *et al.*, 1985) and the cumulative incidence of AIDS has been reported to be as high as 18% (Eyster *et al.*, 1987). Although many HIV-infected haemophilic patients remain well without any clinical symptoms, their clinical prospects remain unclear. The long-term prognosis of HIV-infected haemophilicas has not yet been established, because the time interval from seroconversion to the development of AIDS often exceeds 5 years (Eyster *et al.*, 1985) and because prognostic signs in this patient group have not been clearly determined. Associations have been demonstrated between the duration of seropositivity and decreasing numbers of CD4 lymphocytes and platelets (Eyster *et al.*, 1987). It has also been shown that haemophilic patients with HIV antigenaemia have a significantly higher incidence of AIDS (Allain *et al.*, 1987) and that lack of antibodies to HIV gag (p15, 24 or 55) and/or to pol (p31, 53 or 64) antigens often precedes by 1–4 years the development of AIDS (Ragni *et al.*, 1988). The ability to culture HIV from peripheral blood has also been suggested as an early marker of disease progression in haemophiliacs (Andrews *et al.*, 1987).

We report here a long-term follow up of various immunologic markers and HIV serologic profiles in HIV-infected haemophilic men carried out with the purpose of identifying markers of prognostic value.

Correspondence: Dr Karel Raška Jr., Department of Pathology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane Piscataway, NJ, 08854–5635, USA.

MATERIALS AND METHODS

Patients

The New Jersey Regional Hemophilia Programs' Treatment Center at the Robert Wood Johnson Medical School provides

Table 1. Clinical characteristics of hemophilic patients

Subjects	Age (Years)	Factor consumed (U/year × 10^{-3})	Hgb (g/dl)	Plt ($\times 10^{-3}$/mm^3)	WBC	CD4 (counts/mm^3)	CD8	Months since seroconversion
AIDS ($n=12$)	40±12	55±17	12·3±1·4	211±68	4·3±2·3	102*±201	382†±519	38±14‡
Generalized lymphadenopathy ($n=15$)	30±13	59±60	12·2±2·2	225±74	4·7±2·3	273±209†	632±617§	68±28
Asymptomatic ($n=16$)	36±16	76±106	13·8±2·1	231±70	5·0±1·7	426¶±289	951±515	63±22
HIV seronegative ($n=17$)	27±18	21±26	14·5±2·1	329±9	7·8±2·4	958±661	838±699	—

Data are mean ± 1 s.d. of various parameters. For grouping of subjects, see *Patients*.
* $P<0.005$ as compared with the lymphadenopathy group.
† $P<0.01$ as compared with the lymphadenopathy group.
‡ Median interval from seroconversion to diagnosis of AIDS is shown.
§ $P<0.001$ as compared with the asymptomatic group.
¶ $P<0.0003$ as compared with HIV-seronegative group.

comprehensive care for more than 130 haemophiliacs. Over 60 patients at the Center have participated since 1982 in various studies of immune competence (Saidi, Kim & Raška, 1983; Saidi *et al.*, 1986; Kim *et al.*, 1987). In July 1986, the 60 subjects studied here consented to expansion of the longitudinal study of immunologic parametes to retrospective analysis of HIV serologic markers in the context of a study of the progression of HIV infection. By the end of March 1988, 12 subjects fulfilled revised CDC criteria for AIDS, 15 subjects reached stage III of CDC classification, and 16 HIV-infected haemophiliacs remained asymptomatic (CDC stage II). For comparison, a group of 17 individuals who remain seronegative for both HIV antigen and HIV antibody were included in the study. Of these, only 11 had been exposed to factor VIII concentrate that had not been heat treated. Asymptomatic individuals have been followed regularly at least every 6 months; patients with ARC (CDC stage III) were seen regularly at least every 3 months.

Lymphocyte subset quantifications

The relative numbers of various lymphocyte subsets were quantified by one- and two-colour immunofluorescence after staining with monoclonal antibodies and flow cytometry in a computer-linked Ortho System 50 Cytofluorograf as described earlier (Raskova *et al.*, 1984). Absolute counts were determined from white blood cell and differential counts. Total T lymphocytes were estimated by the antibodies to the CD3 markers, helper/inducer T cells by antibodies to the CD4 marker, suppressor/cytoxic T lymphocytes by antibodies to the CD8 antigen, and B cells by surface immunoglobulin. The number of natural killer (NK) cells was estimated by the Leu 11a antibody. Expression of the interleukin 2 (IL-2) receptor on T cells was estimated by anti-TAC antibody. The functional subsets within CD4 lymphocytes were enumerated by two-colour fluorescence with CD4/4B4 antibodies (inducer of helpers) and CD42H4 antibodies (inducer of suppressors), respectively. Expression of Leu 7 antigen on CD8 cells was quantified by two-colour fluorescence, using Leu 2a/Leu 7 antibodies.

Serologic analysis of HIV markers

Antibodies to HIV-I were tested by Abbott ELISA (Abbott Laboratories, Chicago, IL) and confirmed by a Western blot using Bio-Rad (Bio-Rad, Rockville Centre, NY) strips. Quantitative analysis of antibodies to HIV p24, p31, gp41 and gp120 antigens was performed using the Chiron RIBA-HIV 216 blot (Ortho Diagnostics, Raritan, NJ) that utilized recombinant antigens. Individual antibodies were scored as present when readings of at least 2+ were obtained. HIV p24 antigen was quantified by Abbott or DuPont (Dupont, Wilmington, DE) ELISA techniques. Serologic analysis was performed on frozen plasma specimens that were stored at −70°C.

Statistical analysis

The significance of differences in the absolute counts of lymphocyte subsets was determined by the use of Student's two-tailed *t*-test. The number of patients ranked according to their clinical status with respect to HIV infection and their serologic profile of antibodies to HIV p24 antigen and HIV p24 antigenaemia were compared by Fisher's two-tailed exact test and Kendall's τ *B* ranking test. The prognostic significance of the serologic profile of antibodies to HIV p24 antigen and HIV p24 antigenemia in initially asymptomatic patients over a period of 4 years was also evaluated. The numbers of patients ranked according to their clinical status over the 4 years, and their respective serologic profiles, were compared by Kendall's τ *B* ranking test.

RESULTS

Clinical characteristic of patients

Sixty haemophilic patients were entered into this study; by the end of March 1988, 12 subjects developed AIDS, 15 reached CDC stage III of HIV infection and 16 HIV-infected individuals remained asymptomatic (CDC stage II). Seventeen of the haemophilic patients studied have remained seronegative for HIV antibodies and HIV antigen. Clinical characteristics of the four groups of patients are summarized in Table 1. The consumption of clotting factor concentrate per year in each patient group is shown. Among HIV seronegative haemophiliacs, 11 patients had been exposed to factor concentrate which had not been heat-treated. The most striking differences are seen in the numbers of helper/inducer (CD4) lymphocytes, which were decreased in all three groups of HIV-seropositive patients, in comparison with the group which remained seronegative, or with non-haemophilic healthy controls (883±258 s.d./mm^3). This difference was more pronounced in patients with lymphadenopathy than in asymptomatic HIV-infected

Table 2. Functional lymphocyte subset in haemophilic men

Subjects	CD4	CD8	SIq	Leu 11a	Ia	CD8/Leu7	CD4/4B4	CD4/2H4	TAC
Lymphadenopathy ($n=15$)	273±33*	632±93†	189±32‡	289±70	244±44†	326±70§	88±28	88±23	2·5±0·9
Asymptomatic ($n=16$)	426±42¶	951±87	236±39†	379±52	271±25†	302±46§	147±25	126±24	1·8±0·6
HIV-seronegative ($n=17$)	958±232	838±169	673±120	168±32	654±121	192±80	167±63	424±148	3·3±2·3

Data show absolute cell counts within the lymphocyte cluster of Ficoll-Hypaque isolated peripheral blood mononuclear cells (mean ± 1 s.e./mm^3), except for TAC where the percentage of T-cells expressing the IL-2 receptor is shown. CD4 identified helper/inducer T cells, CD8 suppressor/cytotoxic T cells, SIg identifies surface immunoglobulin positive B cells, Leu 11a shows NK cells. Ia shows cells expressing HLA-DR antigen, CD8/Leu7 shows a subset of CD8 cells which express Leu 7 antigen, CD4/4B4 identifies inducers of helpers and CD4/2H4 inducers of suppressor cells within the CD4 T cell subset. All lymphocyte subsets were enumerated by a single-colour fluorescence, except CD8/Leu7, CD4/4B4 and CD4/2H4 analysed by a two-colour analysis in a computer-linked system 50 Cytofluorograf. Lymphadenopathy identifies patients who reached stage III of the modified CDC classification. Asymptomatic identified haemophiliacs in stage II of CDC classification. HIV seronegative subjects were nonreactive for HIV antibodies and antigen.

* $P<0·01$ as compared to asymptomatic patients.
† $P<0·001$ as compared to asymptomatic patients.
‡ $P<0·002$ as compared to HIV-seronegative patients.
§ $P<0·03$ as compared to HIV-seronegative patients.
¶ $P<0·0003$ as compared to HIV-seronegative patients.

haemophiliacs, but the most striking decrease was seen in the AIDS group. Pairwise differences between the three groups of HIV-infected patients are statistically significant, as is the difference between the asymptomatic HIV-infected group to seronegative and patients (Table 1). Pairwise difference in the other major T cell subset (CD8-suppressor/cytotoxic) were also significant between the three HIV-infected patient groups. In AIDS patients, the absolute CD8 count was lower than control values (507±196 s.d./mm^3). Asymptomatic HIV-infected patients had a significant increase in the number of suppressor/cytotoxic (CD8) lymphocytes in comparison with the other two groups of HIV-infected subjects. The number of CD8 cells in HIV seronegative subjects was increased in comparison with control values ($P<0·001$). Platelet counts in all three groups of HIV-infected haemophiliacs were significantly lower than in the HIV-seronegative group ($P<0·002$). No differences in platelet counts were seen, however, between groups of patients in different stages of HIV infection.

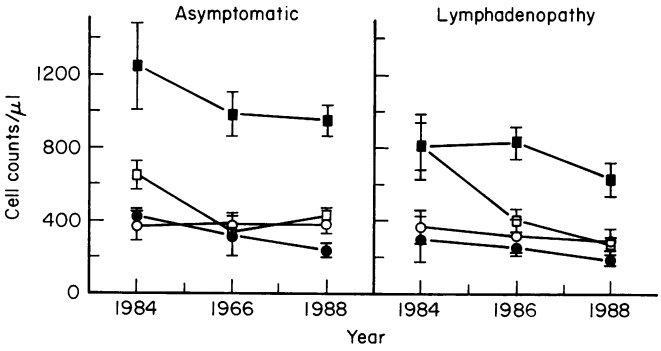

Fig. 1. Changes in functional lymphocyte subsets in HIV-infected haemophiliacs over a 4 year period. The absolute numbers of helper/inducer (CD4), suppressor/cytotoxic (CD8) T lymphocytes, surface-immunoglobulin-bearing B cells (SIg) and NK cells were determined in asymptomatic and lymphadenopathy patient groups over 4 years. Data show mean ± 1 s.e./mm^3. □, CD4; ■, CD8; ●, SIg; ○, NK.

Peripheral blood lymphocytes in haemophiliacs

A detailed analysis of lymphocyte subsets was performed in HIV-infected haemophiliacs who had not developed AIDS, and the results were compared with those in HIV seronegative haemophilia patients (Table 2). HIV-infected haemophiliacs in both the asymptomatic and lymphadenopathy groups had a striking reversal of CD4/CD8 ratio, which was not seen among the non-infected haemophilia patients. Seronegative haemophiliacs had a significantly higher count of B lymphocytes than did the two HIV-infected patient groups ($P<0·002$). The differences in B cell counts between the asymptomatic and lymphadenopathy groups of the HIV-infected patients, however, were not statistically significant. Similar results were seen in the numbers of HLA-DR positive lymphocytes. The differences in the proportion of T cells expressing the IL-2 receptor between the studied groups were not significant. The relative proportions of inducers of helpers (CD4/4B4) and inducers of suppressors (CD4/2H4) within the CD4 T cell subset were comparable between these two HIV-infected patient groups. Among seronegative haemophiliacs, however, there was a higher proportion of the CD4/2H4 cells. The proportion of CD8 cells reactive with Leu 7 antigen was higher in the two HIV-infected groups than in seronegative haemophiliacs ($P<0·03$). In HIV-infected individuals, a more pronounced increase in the proportion of CD8+/Leu 7+ cells was seen in patients with lymphadenopathy than in the asymptomatic HIV-infected patients.

Changes in absolute lymphocyte counts in HIV-infected, AIDS-free subjects over 4 years

Absolute counts of peripheral blood CD4 cells, CD8 cells, mature B cells, and NK cells were followed over a 4-year period in the asymptomatic and lymphadenopathy groups of HIV-infected patients. Results are shown in Fig. 1. Upon entry, all patients studied were asymptomatic. In the lymphadenopathy group there was a continuing decrease of helper/inducer T cell subset (CD4) from 809±180/mm^3 upon entry, through 403±65/mm^3 after 2 years, to 273±33/mm^3 at the end of the study. In the asymptomatic group there was an initial drop over the first 2 years from 646±76/mm^3 to 338±38/mm^3, but no further decrease was seen over the next 2 years (426±42/mm^3). The

Table 3. Antibodies to HIV antigens and p24 antigenemia in HIV-infected haemophilias

Subjects	Antibodies reactive with *				HIV p24 antigen
	p24	p31	gp41	gp120	> 30 pg/ml†
AIDS ($n=12$)	2	11	12	8	10
Lymphadenopathy ($n=15$)	9	14	15	14	5
Asymptomatic ($n=16$)	14	14	16	15	2

* Quantified by blotting using the 'Chiron RIBA-HIV 216' test system which utilizes recombinant antigens. Individual antibodies are shown as present when scores 2+ to 4+ were obtained. The results were verified utilizing standard western blot techniques.

† Quantified using Abbott or DuPont ELISA techniques.

number of CD8 lymphocytes in the lymphadenopathy group was relatively stable during the 4 years, with mean counts of 811 ± 130, 833 ± 90 and 694 ± 92 in years 0, 2 and 4, respectively. In the asymptomatic patients the number of CD8 cells was significantly higher than in the lymphadenopathy group upon entry ($1247 \pm 237/mm^3$), but decreased to $983 \pm 122/mm^3$ after 2 years and remained stable thereafter ($951 \pm 87/mm^3$). The number of B lymphocytes declined slowly, but not significantly, in both patient groups over the study period. There was also no significant change in the numbers of NK cells in the two subject groups.

HIV serologic profile

Of the subjects studied, 17 have remained negative for HIV antibodies and antigen. The other 43 subjects had antibodies to HIV, but only 17 of the 43 HIV-infected haemophiliacs had a sustained p24 antigenaemia (Table 3). Antibodies to individual HIV proteins were quantified with a semi-quantitative blot method using recombinant HIV peptides p24, p31, gp41 and gp120, respectively. The results are shown in Table 3. High levels of antibodies to gp41 glycoprotein were detected in all patients studied, but the pattern of other antibodies varied between the three patient groups. Among patients who developed AIDS, only two out of 12 had detectable antibodies to p24 antigen; and 10 patients had significant HIV p24 antigenaemia (> 30 pg/ml). In the lymphadenopathy group, six out of 15 patients lacked antibodies to p24 antigen and five patients had overt antigenaemia at the end of study. Only two out of 16 subjects who have remained asymptomatic, however, lacked anti-p24 antibodies; those two individuals remained nonreactive to HIV antigen. Sustained antigenaemia was seen in only two asymptomatic subjects, both of whom had significant levels of anti-p24 antibody. This result shows that absence of anti-p24 antibodies and overt HIV antigenaemia are seen most frequently in patients with AIDS (ten out of 12), but in none of the 16 haemophiliacs who remain asymptomatic. The difference between the AIDS and asymptomatic patient groups, in this respect, is statistically highly significant ($P < 0{\cdot}0003$) by Fisher's exact test.

Prognostic significance of serologic pattern

The frozen plasma of 43 HIV seropositive subjects was analysed retrospectively, to establish the date of seroconversion. The mean interval since seroconversion was 68 ± 4 (s.e.) months in the lymphadenopathy group and 63 ± 6 months in asymptomatic individuals. Among patients with AIDS the time interval from seroconversion to the development of AIDS was $37{\cdot}6 \pm 4$ months. Selected specimens from each subject were analysed by quantitative blot for antibodies to p24, p31, gp41 and gp120 antigens and by ELISA for HIV p24 antigen. The initial patterns were established with specimens that were conclusively reactive for HIV antibodies, and were drawn at the time when the patients were clinically asymptomatic with respect to HIV infection. The asymptomatic patients were then classified according to this 'initial' HIV serologic profile into four groups: antibodies to p24 antigen present, p24 antigen absent; antibodies to p24 antigen and p24 antigen both present; antibodies to p24 antigen absent, p24 antigen present; and antibodies to p24 antigen and p24 antigen both absent. The serologic profile for each individual was repeated at least twice over the period of 4 years. Figure 2 shows patterns of four representative patients. Panel a shows an asymptomatic patient with a high titre of IgG against all four HIV antigens tested. Panel b shows a patient who initially had anti-p24 antibodies, but who later lost them, and currently is in CDC classification stage III. Two patients with AIDS, who had low titres of anti-p24 antibody on 'initial profile' are shown in panels c and d. The patient shown in panel d also had a low titre of reactivity with gp 120 antigen. The clinical status of all patients studied was followed for 4 years

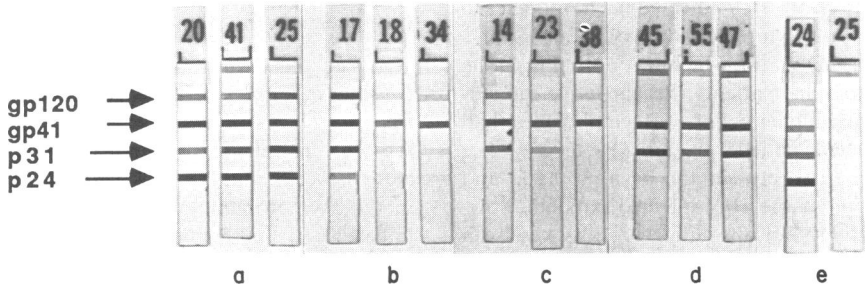

Fig. 2. Quantitative evaluations of antibody patterns in HIV-infected haemophilic patients. Blots of HIV antibodies of four representative patients are shown. The 'initial' blots are in the left lanes; the middle blots show results with specimens obtained 12–24 months later; the right lanes show the blots at the completion of the study. Patients represented: (a) remains asymptomatic; (b) reached CDC stage III in April 1987; (c) fulfilled AIDS criteria in January 1985; and (d) developed AIDS in January 1986. Panel (e) shows positive and negative control blots.

Table 4. Prognostic Significance of antibody to p24 antigen and p24 antigenaemia in infected haemophiliacs

Initial serologic pattern			Clinical staging of patients at yearly intervals											
			1 Year			2 Years			3 Years			4 Years		
Anti-p24	p24 Ag (>30 pg/ml)	Number of Subjects	NS	ARC	AIDS	NS	ARC	AIDS	NS	ARC	AIDS	NS	ARC	AIDS
+	−	24	24	—	—	23	−	1	15	8	1	14	8	2
+	+	1	1	—	—	—	1	—	—	1	—	—	1	—
−	+	12	8	—	4	5	—	7	—	2	10	—	2	10
−	−	6	6	—	—	6	—	—	2	4	—	2	4	—

after establishment of the 'initial' serologic profiles. Results are summarized in Table 4. It is obvious that the slowest progression of the disease was seen in patients who initially had a high titre of anti-p24 antibodies and were p24-antigen-negative. Out of 24 subjects with such serologic profile, 14 remain asymptomatic after 4 years, eight developed ARC, and two have AIDS. The worst outcome was seen in the group who initially lacked antibodies to p24 antigen and were p24-antigen-positive. After 3 years, 10 of those developed AIDS, two developed ARC and none remain asymptomatic. The differences in progression of disease between the above two groups of patients were apparent within 12 months of the 'initial' profile was established ($P < 0{\cdot}003$). The significance of this difference increased thereafter, reaching a statistical level of $P < 0{\cdot}00001$ at the end of 3 years. This result indicates that the serologic pattern of HIV markers provides significant early prognostic information in HIV-infected haemophiliacs, even at a clinically asymptomatic stage of infection.

DISCUSSION

Numerical and functional changes in T lymphocytes in HIV-infected haemophiliacs have been studied at many centres. Progressive depletion of CD4 cells has been noted in those who developed AIDS (Eyester *et al.*, 1985). Moreover, it has been claimed that the decrease in the number of CD4 cells in haemophiliacs correlates with the time interval since seroconversion. A precipitous decrease in the absolute number of CD4 cells usually precedes development of the immunodeficiency syndrome (Eyster *et al.* 1987). Our earlier results confirmed that a striking reduction in the absolute number of CD4 cells preceded development of AIDS (Kim *et al.*, 1987). Results of a 4-year follow-up of 31 initially asymptomatic infected individuals show a significant decrease of CD4 cells during the first 2 years. Our results showed a difference, however, during the next 2 years between individuals who remained asymptomatic (stage II), whose CD4 count stabilized, and those who reached stage III of CDC classification, whose CD4 number further declined. Our results also confirm the earlier report of decreased platelet counts in HIV-infected haemophiliacs (Eyster *et al.*, 1987) in comparison with HIV-seronegative haemophilic subjects.

Earlier reports have indicated that the proportion of $CD8^+$/Leu 7^+ cells increases in haemophiliacs treated with factor VIII concentrate (Ziegler-Heitbrock *et al.*, 1985) and it has been shown that such an increase is associated with HIV infection (Kaplan *et al.*, 1988). Our results confirm these two observations, in that the relative proportion of $CD8^+$/$Leu7^+$ cells is significantly increased in HIV-infected haemophiliacs as compared with the HIV seronegative group. However, our data show that this change also correlates with the clinical status of the HIV-infected haemophiliacs. A greater relative increase was seen in more advanced disease (CDC stage III), in agreement with an earlier report of an increased proportion of $CD8^+$/$Leu7^+$ cells in patients with ARC and AIDS (Stites *et al.*, 1986).

Results in our haemophiliac cohort show that HIV antigenaemia is most prevalent among AIDS patients. Antigenaemia is also more prevalent among patients with lymphadenopathy than among asymptomatic, HIV-infected subjects, despite the comparable time intervals from seroconversion in these two patient groups. This result is in agreement with that reported by Allain *et al.* (1987) who have shown that HIV antigenaemia in haemophiliacs is associated with more severe disease. Similarly, low titres of antibody to HIV p24 antigen were detected more frequently in the AIDS group. On the other hand, all but two patients who remained asymptomatic had high titres of anti-p24 antibody. This result is thus in agreement with the observation that AIDS often developed one to four years after loss of antibody to gag and/or to pol (Ragni *et al.*, 1988). Results reported here indicate that serologic profile of the HIV antibody pattern and of HIV p24 antigenaemia provides important prognostic information in HIV-infected haemophiliacs. The difference in prognosis between asymptomatic patients who were initially 'anti-p24 negative, p24 positive' and those who were 'anti-p24 positive, p24 negative,' is statistically significant within 12 months and reaches the very high level of significance of $P < 0{\cdot}00001$ after 3 years. Indeed, the decrease of anti-p24 antibody and the appearance of persistent HIV antigenaemia have been associated previously with transition to AIDS (Lange *et al.*, 1986). Our studies indicate that this serologic pattern is already a reliable predictor of progression of HIV disease from the early stages of infection. All asymptomatic HIV-infected haemophiliacs who did not have antibodies to p24 antigen and were positive for p24 antigen upon 'initial' analysis of their plasma, progressed to ARC or AIDS within 3 years, whereas only 37% of subjects who lacked anti-p24 antibodies but were not antigenaemic progressed to stage III of CDC classification after 4 years. Of those who initially had a high level of antibody to p24 and were not antigenemic, 58% have remained asymptomatic 4 years after the 'initial' pattern was established. Determination of the HIV serologic profile as well as lymphocyte subset enumerations thus provides early and useful prognostic information in HIV-infected haemophiliacs; it suggests that incorporation of such HIV serologic studies in the long-term follow up and management of HIV-infected patients is warranted.

ACKNOWLEDGMENTS

This work was supported in part by grants from the State of New Jersey (No. 12-87-HEM), the National Institutes of Health (Nos. CA-21196 and AI-25914) and the Hemophilia Association of New Jersey. We wish to thank Dr Stephen M. Shea for critical reading of the manuscript and advice on statistical analysis; Joan Eiesle and Linda Matts for their nursing participation; Michelle Czachur for maintaining the patient data base, Margaret Czarnecki for flow cytometry and Renee Hart for serologic assays. Some of the data in this study are a part of the honors dissertation of K. Raška III, in partial fulfillment of requirements for the M.D. degree at the Oliver Wendell Holmes Society, Harvard Medical School, Boston, MA.

REFERENCES

ALAIN, J.P., LANRIAN, Y., PAUL, D.A., VERROUST, F., LEUTHER, M., GAZENGEL, C., SENN, D., LARRIEU, J.M. & BOSSER, C. (1987) Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. Potential clinical importance. *N. Engl. J. Med.* **317,** 1114.

ANDREWS, C.A., SULLIVAN, J.L., BRETTLER, D.B., BREWSTER, F.E., FORSBERG, A.D., SCESNEY, S. & LEVINE, P.H. (1987) Isolation of human immunodeficiency virus from hemophiliacs: correlation with clinical symptoms and immunologic abnormalities. *J. Pediatr.* **111,** 672.

CENTERS FOR DISEASE CONTROL (1986) Surveillance of hemophilic-associated acquired immunodeficiency syndrome. *MMWR* **35,** 669.

EYSTER, M.E., GAIL, M.H., BALLARD, J.O., AL-MONDHIRY, H. & GOEDERT, J. J. (1987) Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T-cell subsets, platelet counts, and age. *Ann. intern. Med.* **107,** 1.

EYSTER, M.E., GOEDERT, J.J., SARNGHADHARAN, M.G., WEISS, S.H., GALLO, R.C. & BLATNER, W.A. (1985) Development and early natural history of HTLV-III antibodies in persons with hemophilia. *JAMA* **253,** 2219.

HILGARTNER, M.W. (1987) AIDS and hemophilia. *N. Engl. J. Med.* **317,** 1153.

JOHNSON, R.E., LAWRENCE, D.N., EVATT, B.L., BREGMAN, D.J., ZYLA, L.D., ALEDORT, L.M., EYSTER, E., BROWNSTEIN, A.P. & CARMAN, C.J. (1985) Acquired immunodeficiency syndrome among patients attending hemophilia treatment centers and mortality experience of hemophilics in the United States. *Am. J. Epidemiol.* **121,** 791.

KAPLAN, J., SARNIAK, I., LUSHER, J. & THE TRANSFUSION SAFETY STUDY GROUP (1988) Increase in Leu 7+ lymphocytes in HIV I-seropositive patients with hemophilia repeatedly treated with clotting factor concentrates. *Clin. Immunol. Immunopathol.* **46,** 337.

KIM, H.C., NAHUM, K., RAŠKA, K., GOCKE, D.J., KOSMIN, M., KARP, G.I. & SAIDI, P. (1987) Natural history of acquired immunodeficiency syndrome in hemophilic patients. *Am. J. Hematol.* **24,** 169.

LANGE, J.M.A., PAUL, D.A., HUISMAN, H.G., DE WOLF, F.J., VAN DEN BERG, H., COUTINHO, R.J., DANNER, S.A., VAN DER NOORDAA, J. & GOUDSMIT, J. (1986) Persistent HIV antigenemia and decline of HIV core antibodies associated with transition to AIDS. *Br. Med. J.* **293,** 1459.

RAGNI, M.V., O'BRIEN, T.A., REED, D., SPERO, J.A., & LEWIS J.H. (1988) Prognostic importance of antibodies to human immunodeficiency virus of recombinant immunoassay and Western blot techniques in HIV antibody-positive hemophiliacs. *AIDS Res. Hum. Retroviruses* **4,** 223.

RASKOVA, J., GHOBRIAL, I., EISINGER, R.P., SHEA, S.M. & RAŠKA, K. JR. (1984) Suppressor cells in end stage renal disease: functional assays and monoclonal antibody analysis. *Am. J. Med.* **76,** 847.

SAFAI, B., SARNGHADHARAN, M.G., GROOPMAN, J.E., ARNETT, K., POPOVIC, M., SLISKI, A., SCHUPBACH, J. & GALLO, R.C. (1984) Seroepidemiologic studies of human T-lymphotropic retrovirus type III in AIDS. *Lancet*, **i,** 1438.

SAIDI, P., KIM, H.C. & RAŠKA, K. JR. (1983) T cell subsets in hemophiliacs. *N. Engl. J. Med.* **308,** 1991.

SAIDI, P., LEGA, B.Z., KIM, H.C. & RAŠKA, K. JR. (1986) Effect of Danazol in clotting factor levels, bleeding incidence and immune parameters in hemophiliacs. *Blood,* **68,** 673.

STITES, D.P., CASAVANT, C., MCHUGH, T.M., MOSS, A.R., BEAL, S.I., ZIEGLER, J.G., SAUNDERS, A.M. & WARNER, N.I. (1986) Flow cytometric analysis of lymphocyte phenotypes in AIDS using monoclonal antibodies and simultaneous dual immunofluorescence. *Clin. Immunol. Immunopathol.* **38,** 161.

ZIEGLER-HEITBROCK, H.W.L., SCHRAMM, W., STACHEL, D., RUMPOLD, H., KRAFT, D., WERNICKE, D., VON DER HELM, K., EBERLE, J., DEINHARDT, F., RIEBER, E.P. & RIETHMÜLLER, G. (1985) Expansion of a minor subpopulation of peripheral blood lymphocytes ($T8^+$/$Leu7^+$) in patients with haemophilia. *Clin. exp. Immunol.* **61,** 633.